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Changes in total protein, Agglutinin, TFAA and proline in the hemolymph of *Penaeus indicus* H. Milne Edwards during mud bank formation

S. Jayasree* and G. S. Selvam

Biochemistry & Genetic Engineering Research Unit, Department of Biotechnology, Cochin University of Science & Technology, Cochin 682 022.

Abstract

Total protein, agglutinin, total free amino acid and proline contents in the hemolymph of *Penaeus indicus* collected from a mud bank along the coast of Kerala, were estimated quantitatively and compared with the samples collected from non-mud bank area at the same time. Total protein, agglutinin, total free amino acid and proline contents were found significantly higher ($P<0.001$) compared to non-mud bank sample. Changes in the biochemical parameters of the hemolymph in this prawn from mud bank area reflect specialisation and adaptation to cope with the changing stressful environment. The concentration of agglutinin or proline can be used as an index for monitoring health condition of prawns experiencing environmental stress, pollution, disease or toxicity etc.

Members of the genus *Penaeus* distributed over a wide range of salinities are cultured under a variety of conditions in many tropical and subtropical areas of the world (Cawthrone *et.al.*, 1983). Many fundamental features of the class Crustacea are reflected in the nature of the internal medium, the hemolymph. The hemolymph was chosen as a biological agent susceptible and modified by the pollutants. Euryhaline shrimps adapt physiologically to the alteration in the surrounding medium. These changes are most obviously manifested in the composition of the hemolymph (Pequeux, 1995). Solute concentration in the hemolymph is a function of solute concentration in the medium as well as molt stages (Mantel and Farmer, 1983). Thus, hemolymph composition of shrimps will provide indication about the physiological modifications associated with molting process, developmental stages, defense mechanism and environmental stress.

For the present study, hemolymph was extracted from the prawns collected during the formation of mud bank. Mud bank is a calm, undisturbed semicirular area of sea water boarded by disturbing waves, formed during the wake of south-west monsoon along the Kerala coast. This is a unique phenomenon elsewhere noticed in the world (Kurian and Sebastian, 1986). Mud bank is rich in organic load, clay, macro and micro fauna that form the food of crustaceans, especially prawns. Mud bank area is ruch in prawn fishery (Nair, 1983). Considering the paucity of information on the hemolymph parameters in relation to environmental stress, it was decided to undertake the present investigation in *Penaeus indicus*, subject to different environmental conditions during mud bank formation.

* *Present address : Department of Zoology, Mercy College, Palghat 678 006, Kerala*

Material and methods

P. indicus of body length 130 mm were collected from mud bank and non-mud bank area of Vadanappilly near Chettuva, Kerala during south - west monsoon period of July - August 1997.

Hemolymph was withdrawn from the pericardial sinus with No. 24 hypodermic needle and transfered to a sterile vial with anticogulant 3% ($^{w}/_{v}$) sodium citrate (9:1). The hemolymph was allowed to clot at 4°C for 12 hrs. The supernatant (serum) collected after centrifugation at 5,000 rpm for 15 min. was used for biochemical analysis of total protein, agglutinin, total free amino acid (TFAA) and proline using standard procedures. Total serum protein was estimated as mg/ml by the method of Lowry *et al.* (1951).

P. indicus serum was separated into fractions using Sephadex G-200 column. The 7th fraction with agglutinating property with bacterial cells were precipitated out at 45% saturation of ammonium sulphate at 4°C for 1-2 hrs. The precipitate was separated after centrifugation at 5,000 rpm for 20min. at 4°C and dissolved in 0.85% NaCl. This agglutinin solution after dialysis against distilled water was used for biochemical studies. The concentration of agglutinin solution was determined as µg/ml following the method of Lowry *et al.* (1951).

Total free amino acid concentration in the serum as mg/ml was calculated by Ninhydrin method of Yemm and Cocking (1955) and Sadasivam and Manikam (1992). Proline content in the serum was estimated as µmol/100 ml using the method of Sadasivam and Manikam (1992). One-way analysis of variance (ANOVA) was used to determine the level of significance between locality (Ross, 1987).

Results

The hemolymph of prawn collected from mud bank recorded maximum concentration of total protein, agglutinin, total free amino acid and proline (Table 1). Significant variation in all the parameters were observed between mud bank and non-mud bank area ($P<0.001$). Agglutinin concentration of 3.47µg/ml and 3.31 and 3.31µmol/100ml of proline from the hemolymph of mud bank sample indicated the high stressful environment during the mud bank formation. Relative increase in protein and free amino acid concentration was also observed.

Discussion

The chemical composition of the hemolymph in Crustacea depends more or less directly on the nature of environment. The chemical composition varies with respect to the environment (Florkin and Scheer, 1971). Increase in protein con-

Table 1. *Concentration of biochemical parameters (Protein, Agglutinin, TFAA and Proline) vs. Locality*

Parameter	Locality +	
	Mud bank	Non-mud bank area
Total protein (mg/ml)	119.06±0.628	108.91±0.293
Agglutinin (µg/ml)	3.47 ± 0.223	0.97±0.117
Total FAA (mg/ml)	9.31±0.556	3.21±0.18
Proline (µmol/100ml)	3.31 ± 0.138	1.55±0.186

† mean of triplicate analysis. *P<0.001

centration observed from mud bank sample may be due to the intake of protein rich food items from the mud bank area. Protein concentration in the hemolymph was affected by nutritional state as well as dietary source (Hepper, 1978; Ferraris *etal.*, 1986). Significant increase of agglutinin ($P<0.001$) concentration observed in mud bank sample may be due to abundance of microorganisms. Agglutinin synthesis can be stimulated by changes in salinity, environmental stresses such as temperature, pathogens or pollution resulted during the formation of mud bank (Nair, 1983; Kurian and Sebastian, 1986). Agglutinin was considered as one among the hemolymph natural defense protein capable of agglutination, hemolysis and antibacterial properties, was isolated and charachterized to homogeneity by Jayasree (1999). Agglutinin was a multifunctional protein of molecular weight 181 kDa with two subunits 97 kDa and 84 kDa respectively, fight against potential pathogens. An ELISA was developed by Jayasree and Selvam (1998) to quantify the agglutinin concentration in the hemolymph. The stress conditions generated in the mud bank augment the agglutinin synthesis from the hemocytes in crustaceans. Increase in metallo - protease secretion was noticed in *Cryptobia salmositica* living in the medium with pathogens (Xzuo and Woo, 1998). The TFAA and proline concentration exhibited variation between the samples collected from mud bank and non-mud bank area. The increase of free amino acids may be due to low salinity of mud bank area consequent to the inflow of fresh water during heavy monsoon. Free amino acid content in the hemolymph also depends on the composition of food and the physiological state of the animal (Dean and Scott, 1965). Increased titer of FAA in the hemolymph also depends on the composition of food and the physiological state of the animal (Dean and Scott, 1965). Increased titer of FAA in the hemolymph of prawn collected from mud bank may be due to the abundance of food materials (Damodaran, 1973). The stress mediated while mud bank formation may be the reason for the increase of proline concentration in sample. Proline is a basic amino acid that can be used as an indicator of external environmental changes. Methyl fernesoate (MF) in the hemolymph, like proline was found to increase in the hemolymph followed by physiological stress such as increased temperature, anoxia and decreased salinity in the green crab, *Carcinus maenas* (Lovett, 1997).

Significant increase of total protein, agglutinin, TFAA and proline in the mud bank sample compared to non-mud bank area establishes the homeostasis maintained by prawns to cope with the surrounding stressful condition. These parameters can be used as an index for monitoring the health conditions of prawns, which are susceptible to a variety of environmental stresses such as pollution, toxicity, diseases etc.

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